

October 08, 2013

Dear Editor,

Please find enclosed the revised version of our manuscript.

Title: Blood groups, hemoglobin phenotypes and clinical disorders of consanguineous YANSI population

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The comments and concerns raised by the Reviewers were extremely helpful in improving the manuscript. A detailed response to the Reviewers is enclosed below.

The paper is co-authored by scientists with a professional knowledge of the English language and who have thoroughly revised the manuscript. We believe that the language of the manuscript reaches Grade A.

All authors have reviewed the manuscript and agree with its contents.

Response to the Reviewers

Reviewer 00506058

We thank you for your appreciation of our work.

Reviewer 02512574

We thank you for your comments.

- (1) Please provide more information on the controls: Were they of the same race, same region, same country etc.?**

We added the following paragraph to the Materials and Methods paragraph: All our samples were collected in the Bandundu province of Democratic Republic of Congo. The Yansi subjects were located in the district of Kwilu where the Yansi people live whereas control subjects were located in the city of Kikwit also in the Bandundu province. Controls subjects did not belong to the Yansi tribe but lived in the same region of Congo.”

- (2) The G6PD deficiency statistics should be given separately for men and for women.**

Table 3 shows the G6PD deficiency data separately in men and women.

- (3) The blood grouping and extended phenotyping procedures' methods are inadequate and unreferenced. What technique was used to determine the antigen antibody reaction? Which manufacturer's antibodies were used?**

We added the following paragraph to the Materials and Methods paragraph: “The tube technique was used for all analyzes of blood grouping using monoclonal and polyclonal reagents in accordance with manufacturer’s procedures. The phenotypes ABO and D were performed by the double method of Beth-Vincent and Simonin. The ABO and D antigens were typed using monoclonal antisera of Pelikloon (Sanquin). Typing of C, c, E, e, Fya, Fyb, Jka, Jkb, M antigens was carried out with antisera from Immundiagnostika (Seraglu) while K, N, S, s antigens were typed using antisera from Seraclone (Biotest).

We have also added the following reference: Chiaroni J, Roubinet F, Bailly P, Mannessier L, Noizat-Pirenne F. Les analyses immunohématologiques et leurs applications cliniques. France: John Libbey Eurotext, 2012: 39-66.

- (4) For G6PD deficiency the SpotTest technique was used, which appears to be a tradename of a particular kit. However, the reference 21. points to a 1968 article. Please provide at least minimal information about the kit you used, including its sensitivity, principle etc.**

G-6-PD activity was measured by the Beutler fluorescent spot test technique. The principle of this test is based on the visualisation of NADPH produced in a blood spot sample. The reagent is prepared by mixing glucose-6P, β -nicotinamide adenine dinucleotide phosphate, saponine, Tris HCl and glutathione (all from Sigma). The reaction is observed under a UV lamp on Greiner Plate 655191.

- (5) Of the disorders studied, were any correlations observed between sickle cell trait (AS) and thrombotic diseases, epilepsy, pregnancy losses etc?**

In this study we have not highlighted a correlation between sickle cell trait and the above-mentioned diseases. However, this is a track for future research in this population.

Reviewer 00225291

We thank you for your comments and remarks.

- (1) Text is well written, although some corrections are needed, such as in the first paragraph of the MATERIAL AND METHODS SECTION: "...of the Congo, where live the Yansi people" should be "...of the Congo, where the Yansi people live"**

Corrections were made accordingly.

- (2) The main result is a difference in the frequency of some blood antigens between both groups. With regard to other comparisons, there are not differences and the paper should then focus mainly on the significance differences**

In the numerous criteria reported in this study, both similarities and differences were observed between Yansi and controls. We discussed the differences but also wished to report the overall data since these can be referenced and challenged in further studies in the same tribe.

- (3) Authors should provide more data on the control population: is the geographic area of recollection of samples close to the Yansi tribe? This demographic data are relevant**

The Yansi subjects were located in the district of Kwilu whereas control subjects were located in the city of Kikwit, both in the Bandundu province. Controls subjects did not belong to the Yansi tribe but lived in the same region of Congo.

(4) Authors the end up commenting the “...medical risks associated with consanguineous unions...”, but this already known


It is true that medical risks associated with consanguinity are known. In this study we identified these risks in the Yansi population particularly. Few documented studies have been conducted in this rural area and thus we feel that our data are worthwhile to help in sensitizing the population on these risks.

(5) Finally, authors stress the relevance of their findings with regard to clinical aspects, but none of the results shown support their conclusions. For instance, the increased abortion rate in the Yansi tribe may well be due to HLA similarity between spouses, as already shown by J. Dausset in other consanguineous tribes some years ago. I do not doubt the clinical problems exist, but with the results shown, it cannot be deduced there is a direct causal link.

We agree with this Reviewer's comment, which we added in the conclusion. We recommend newborn screening of a larger cohort to get more clarification on the effects of consanguinity on the phenotypes studied in the present works. In addition, clinical disorders potentially linked to consanguinity can also be exacerbated by socio-economic factors.

I am looking forward to hearing from you at your earliest convenience.

Sincerely yours,



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